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Microbiological Assessment of Smoked Fish Marketed in Daloa: Herring "*Clupea harengus*", Horse Mackerel "*Trachurus trachurus*", and Atlantic Mackerel "*Scomber scombrus*"

Ibourahema COULIBALY[1,*], Adja Mansagna COULIBALY[1,3], Foba Stéphane Isaac FOBA[1], Kouakou Ahossi KONAN[2], Kra Athanase KOUASSI[1], Ibrahim KONATÉ[2]

[1]Agrovalorisation Laboratory, Agroforestry Training and Research Unit (UFR-AGRO), University Jean Lorougnon GUEDE PB-150, Daloa, Ivory Coast
[2]Training and Research Unit in Agroforestry, Laboratory of Host-Microorganism Interactions, Environment and Evolution (LIHME), Jean Lorougnon Guédé University, Daloa, Côte d'Ivoire
[3]Laboratory of the Unit of Antibiotics, Natural Substances and Surveillance of Resistance of Microorganisms to Anti-Infectious (ASSURMI), Pasteur Institute of Ivory Coast (IPCI) BP-490, Abidjan-01, Ivory Coast
*Corresponding author: ibourahema.ujlog@email.com

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Abstract - Smoked fish are a staple protein source in Côte d'Ivoire but are frequently associated with microbial contamination, which can pose serious health risks. This study investigated the microbiological quality of three commonly consumed smoked fish species, herring (*Clupea harengus*), horse mackerel (*Trachurus trachurus)*, and mackerel (*Scomber scombrus*), sold in Daloa. Sampling was conducted across five markets between December 8. 2021, and January 13. 2023. Microbial analyses were performed using standard culture methods with serial dilutions to quantify key pathogenic bacteria, including Salmonella spp., Staphylococcus aureus, and Escherichia coli. The results indicated widespread contamination in most samples. *Salmonella* spp. were present in nearly all fish, except horse mackerel from Lobia market and mackerel from Orly market. Average microbial loads were 0.89.105 CFU/g for horse mackerel, 0.66.105 CFU/g for herring, and 9.3.104 CFU/g for mackerel. All samples were contaminated with *Staphylococcus aureus*, with the highest levels observed in horse mackerel from Lobia market (0.77.107 CFU/g). Additionally, *E. coli* was detected across several samples, suggesting fecal contamination at different stages of processing or handling. These findings emphasize the high prevalence of pathogenic bacteria in smoked fish marketed in Daloa, underscoring the need for stricter hygiene measures during processing, storage, and sale. Addressing these contamination risks is crucial for protecting public health and ensuring the safety of smoked fish as an essential dietary component.

Keywords: *fish, mackerel, horse mackerel, herring, Daloa, smoke*

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1. Introduction

Capture fisheries and aquaculture together supply about 143 million tons of fish for human consumption worldwide [1]. Aquaculture alone contributes nearly 76% of global freshwater fish production [2]. In many African countries, as in other regions, fisheries and aquaculture remain a major source of animal protein for local populations [3]. In West Africa, fish and fish-based products such as surimi, soups, and croquettes are integral to the diet [4]. Beyond their dietary importance, fish provide essential proteins, vitamins, and minerals that help compensate for nutritionally unbalanced diets. In Côte d'Ivoire, fish is the most important source of animal protein, with an estimated national consumption of 320,000 tons per year, while local production accounts for only about 42,000 tons. This gap creates a heavy reliance on imports to satisfy demand [5]. At the same time, fisheries play a critical socio-economic role, generating nearly 70,000 direct jobs and supporting more than 400,000 people [6]. Fish consumption accounts for approximately 50% of animal protein intake among Ivorian consumers, with an average of 15–16 kg per person annually [7]. However, national fish production

remains around 50,000 tons against a demand exceeding 360,000 tons, most of which is covered by imports valued at nearly 289 million euros, resulting in a significant trade imbalance [8,9,10]. Despite its nutritional and economic importance, fish is highly perishable. In Côte d'Ivoire, particularly in Daloa, smoking represents the most common preservation technique. This method, largely artisanal, is mainly carried out by small-scale processors. Poor hygiene practices during processing and inadequate storage conditions frequently compromise product safety, creating favorable conditions for microbial contamination. As a result, smoked fish can serve as a vehicle for foodborne pathogens [6]. Foodborne infectious diseases, often associated with microorganisms present in smoked fish, represent a major public health concern and impose a socio-economic burden through human suffering and reduced productivity [7]. Ensuring the microbiological safety of smoked fish is therefore essential. Pathogenic microorganisms and chemical contaminants have been identified in smoked fish products and constitute a growing public health issue [11]. The risks associated with these products highlight the need to assess and control microbiological hazards to guarantee consumer protection. From a nutritional standpoint, fish are valuable sources of high-quality proteins, lipids, vitamins, and minerals, with fatty species rich in omega-3 polyunsaturated fatty acids attracting particular attention due to their recognized health benefits [12,13]. Horse mackerel (*Trachurus* spp.) and Atlantic mackerel (*Scomber scombrus*) are among the most consumed species in West Africa, appreciated both for their nutritional composition and sensory qualities. Horse mackerel, a pelagic fish of the Carangidae family, is characterized by a curved lateral line with bony scutes, silvery flanks, and a distinctive black spot near the gill cover. Being a fatty fish, it is rich in omega-3 fatty acids, with fat content peaking in autumn [12]. Its firm muscle texture and silvery appearance enhance its culinary and commercial value. Atlantic mackerel, a semi-fatty pelagic fish of the Scombridae family, is recognized by its fusiform body, striped dorsum, metallic flanks, and two dorsal fins, one with spiny rays [14]. Lacking a swim bladder, it is adapted for continuous swimming. Like horse mackerel, it is rich in omega-3 fatty acids and provides B vitamins, vitamin D, selenium, and iron, making it highly nutritious [13]. Although these species are widely consumed, information on their microbiological quality, spoilage dynamics, and the impact of processing and storage conditions remains limited. A better understanding of their physicochemical and nutritional characteristics is therefore crucial for optimizing preservation methods and ensuring product safety. In this context, the present study aims to evaluate the microbiological quality of three smoked fish species, herring (*Clupea harengus)*, horse mackerel (*Trachurus trachurus)*, and Atlantic mackerel (*Scomber scombrus*)—sold in Daloa, Côte d'Ivoire. The specific objectives are to: (i) determine the presence of indicator and pathogenic microorganisms, including *Salmonella* spp., *Staphylococcus aureus*, and *Escherichia coli*; (ii) identify microbiological risks associated with the consumption of these smoked fish; and (iii) assess processing and storage conditions that may favor microbial proliferation.

2. Materials and Methods

2.1. Materials

2.1.1. Study Area

The study was carried out in the locality of Daloa, precisely in the various markets surveyed. The city of Daloa is located in the Haut-Sassandra region in the central-western part of Côte d'Ivoire between 6° and 7° north latitude and between 7° and 8° west longitude (Figure 1). Five (5) different markets were investigated for the surveys (Lobia, Orly, Kennedy 1 and 2, Abattoir 1 and 2 and the single market). This region contributes to the country's wealth with the marketing of smoked fish because of the number of vendors surveyed on the one hand and the participation of these vendors in the country's economy on the other.

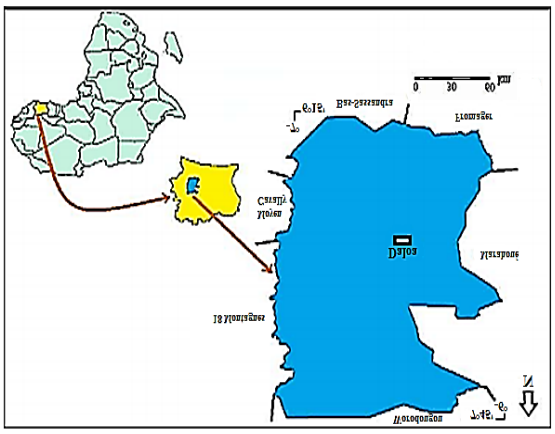

Figure 1. Location of the study area

2.1.2. Biological Material

The biological material used in our study consists of smoked fish that were purchased from different vendors in three (3) markets in Daloa (Grand Market; Lobia; Orly) and put in Stomacher sachets and then sent directly to the laboratory for analysis.

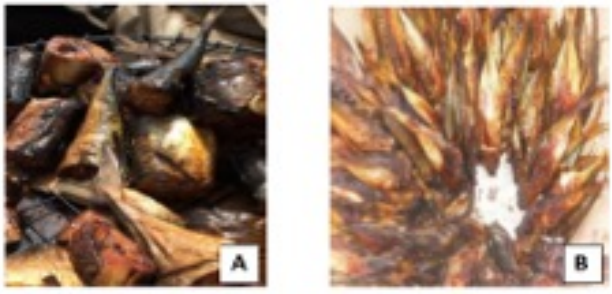

Figure 2. Smoke fishes (A: mackerel and haring; B: horse mackerel)

2.2. Methods

2.2.1. Investigation

For the conduct of the surveys, two types of survey sheets were produced: - a first sheet focused on the marketing of traditionally smoked fish in five (5) markets in the city of Daloa, in particular on the vendors. The

essential information provided was the age range, the types of fish sold, the type of fish sold the most, the level of education, the different ethnic groups, the method of conservation and the sources of supply. The survey took place from 08-12. 2021 to 13. 01. 2023 in five (5) markets in the city of Daloa (the Orly market, the Big market, the Lobia market, the slaughterhouse market 1 and 2 and the Kennedy market 1 and 2). In total, the investigation involved 80 smoked fish sellers; a second sheet provided information on consumers. The main information sought included the age range, the types of fish consumed and among these the most consumed type of fish, the level of education of consumers, the symptoms encountered after consumption and the methods of cooking smoked fish. The survey took place from 15. 01. 2023 to 17. 02. 2023 in the five (5) markets of the city of Daloa The respondents were of both genders, from all social strata and all levels of education. In total, the survey involved 150 smoked fish consumers.

2.2.2. Sampling

In order to monitor the quality of the fish from the reception of the fresh fish to the obtaining of the smoked fish and their sale, three specific markets (Lobia, Orly, Grand Marché) among the five (5) markets surveyed in the city of Daloa were chosen for sampling. A total of 27 samples of smoked fish randomly taken from smokers, including nine (9) fish samples in the Lobia market (3 mackerel, 3 herring and 3 horse mackerel); nine (9) others in the Orly market (3 mackerel, 3 herring and 3 horse mackerel) and nine (9) in the large market (3 mackerel, 3 herring and 3 horse mackerel) were analyzed. Samples were collected for microbiological analysis under aseptic conditions: sterile latex gloves are used to protect the hands during sampling. These fish samples are collected and packaged in sterile bags and sent to the Laboratory of Host-Microorganism Interactions and Evolution (LIHME) of the Jean Lorougnon Guédé University for microbiological analyses.

2.2.3. Pathogen testing

2.2.3.1. Detection of suspected pathogenic *Staphylococcus aureus* [15]. (Standard NF V 057-1 - January 2004)

The culture medium of choice used for this research is Baird-Parker (BP), with the addition of a mixture of egg yolk with potassium tellurite + sulphamethazine 0.2% (antibiotic)), 0.1 mL of the stock suspension or dilutions retained is removed and then spread with a sterile spreader on the surface of the agar already poured and solidified in a sterile petri dish. After the cans around the bunsen burner have solidified, they are incubated at 37 °C for 48 hours. A first reading is made after 24 hours; a second after 48 hours of incubation. Characteristic black, shiny, domed colonies surrounded by a white precipitate and a lightening halo are counted. Only boxes with between 15 and 150 are considered.

2.2.3.2. *Salmonella* spp. testing (NF EN ISO 6579/A1 standard: July 2007) [16].

The search for Salmonella was carried out in accordance with the NF EN ISO 6579 standard. The analysis was carried out in several successive stages: pre-enrichment to recover stressed bacteria, followed by selective enrichment to promote the multiplication of Salmonella compared to the competing flora. This phase was continued by isolation on specific selective media, followed by biochemical identification in accordance with the methods described by Colin (2002) [17]. and Bonny et al. (2011) [18].

Pre-enrichment consisted of using 25 g test portions, each suspended in 225 ml of buffered peptone water and then incubated at 37 °C for 18 to 24 hours. Selective enrichment was performed only on the Rappaport Vassiliadis (RV) medium. For this purpose, 0.1 ml of the pre-enriched stock solution was transferred to a tube containing 10 ml of pre-prepared and sterilized RV. After homogenization, the tube was incubated at 44°C for 24 hours.

From the incubated enrichment culture, Salmonella isolation was performed on Hecktoen agar. After stirring the culture in the Rappaport Vassiladis medium, a drop was taken with a sterile platinum loop and then streaked on the surface of a petri dish containing Hecktoen agar. Incubation was carried out at 37°C for 24 hours, or up to 48 hours in the absence of characteristic colonies after the first incubation. On this medium, the typical colonies observed were in the form of green or blue colonies with a black center.

2.2.3.3. Search for *Escherichia Coli*

A volume of 0.1 mL of the 10-1, 10-2 and 10-3 dilutions is taken using a sterile pipette and then placed in a pre-poured sterile Petri dish (containing Rapid medium. E. coli). This operation is repeated in order to obtain for each dilution two Petri dishes, i.e. two tests. After solidification around the bunsen burner, the cans are turned over and incubated at 44 °C for 24 to 48 hours ± 1 hour. After the incubation period, the bluish colonies between 15 and 150 characteristic colonies are counted.

2.2.4. Enumeration

After the incubation period specified in the sprout-specific standard, the characteristic colonies shall be counted for each box containing less than 300 colonies and a minimum of 15 colonies or any other number specified in the standard. The calculation of the number of microorganisms per milliliter of sample (CFU/mL) from the number of colonies obtained in the chosen Petri dishes is performed by the equation described by [19].

$$N = \frac{\sum C_i}{\left(\left(N_1 + 0{,}1N_2\right) d.V\right)} \quad (1)$$

ΣCi: Sum of the characteristic colonies counted on all the boxes retained;
N_1: Number of boxes retained at the first dilution;
N_2: Number of boxes retained at the second dilution;
d: Dilution ratio corresponding to the first dilution;
V: Seeded volume (ml);
N: Number of microorganisms (CFU/mL).

2.2.5. Statistical Analyses

The results of the surveys were entered into Excel 2013 and the statistical analysis was performed using SPSS 17.0

software. The results were analyzed by the variance method (ANOVA) using the STATISTICA 7.1 software. The comparison of the means is carried out by testing the smallest significant difference LSD (Least Significant Difference). This method of analysis consists of looking for averages that differ significantly from each other. The differences are significant when P<0.05. These materials and methods have enabled us to obtain the following results, which will be discussed.

3. Results

3.1. Characteristics of Fish Smokers and Diagnosis of the Different Types of Smoking

The survey revealed that among the vendors surveyed, there are several ethnic groups and the majority are made up of kôyaka (34 %) followed by mahouka (21 %), odienneka (18 %), yacouba (5 %), gueré (3 %), and gouro (4 %). These smoked fish sellers are in the age group of 20-50 years old with an average age of 35 years old. In the five (5) markets surveyed, 98.75 % of the respondents are women and only 1.25 % are men; 85 % of these women are illiterate, 10% have completed primary school and 5% secondary school. All the vendors use the traditional smoking method and mainly sell their product in the various markets in the city of Daloa. Nearly 46 % of these vendors mainly smoke mackerel fish *(Scomber scombrus*), 30 % for the smoking of thawed herring fish (*Clupea harengus*); 18 % for the smoking of frozen horse mackerel fish (*Trachurus trachurus)* and 7% for the smoking of other types of fish. In addition, the vendors met in the markets have no knowledge of hygiene (92.5%), only 7.5% have received hygiene training (Table 1).

Table 1. Characteristics of the saleswomen interviewed

	Distribution			
	Characteristics	Number		Percentage (%)
Sex	Men	1	80	1.25
	Women	79		98.75
Education's level	None	68	80	85.00
	Primary	8		10.00
	Secondary	4		5.00
Hygiene's concept	Hygiene training	6	80	7.5
	No training	74		92.5

3.2. Main Types of Smoked Fish Sold in the Different Markets of Daloa

Among the five markets surveyed, horse mackerel is the least sold fish, unlike the two types of fish. Mackerel fish is sold in large quantities in the markets (Grand Marché, Lobia and Kennedy) while it is sold in small quantities in the abattoir and Orly markets. On the other hand, herring fish is sold more in these two markets (Orly and slaughterhouse) and sold in insufficient quantities in the markets of Lobia, Grand Marché and Lobia. Thus, mackerel fish remains the most distributed fish with a high sales volume of thirteen (13) in the large market, followed by herring fish which is sold in average quantities with nine (9) in the Lobia market and finally horse mackerel fish distributed in insufficient quantities with four (4) as sales outlets in the slaughterhouse market. The most distributed fish in the markets: - the slaughterhouse market: herring fish, - at the large market and the markets of Kennedy and Lobia: mackerel fish, - At the Orly market: herring fish, (Figure 3).

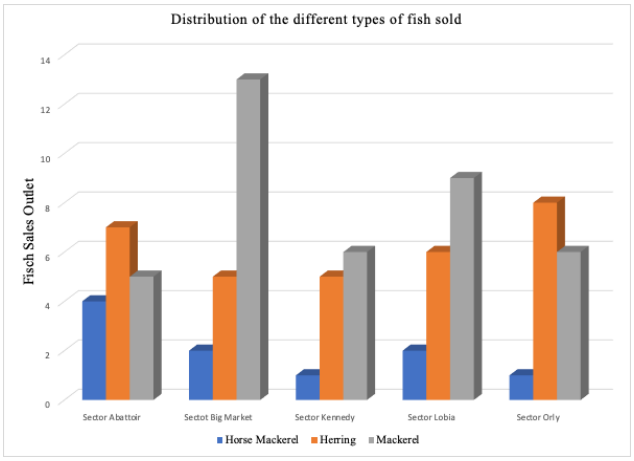

Figure 3. Distribution of different types of fish sold

3.3. The Types of Fish Consumed in the Different Markets Surveyed

The results of consumer surveys have shown that regardless of the type of fish, women are the biggest consumers compared to men. Also, mackerel fish is the most consumed compared to the other two varieties of fish, followed by horse mackerel and finally herring fish regardless of sex (Figure 4)

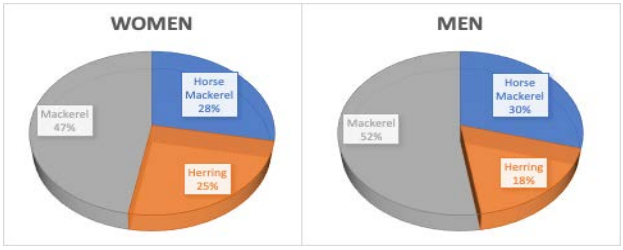

Figure 4. List of types of fish consumed

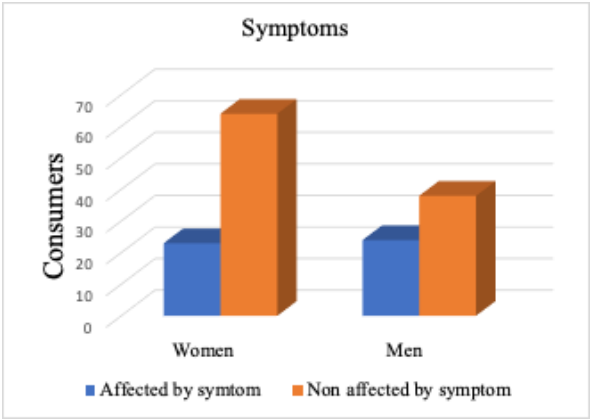

Figure 5. Symptoms encountered after consumption

The consumer survey reveals that among people who do not experience a symptom (no), women are the ones who experience more of a health problem after consuming the types of smoked fish, unlike men who are the least affected. In addition, the number of women and men experiencing symptoms (yes) is almost identical (Figure 5).

3.3.1. Staphylococcus Aureus

The microbiological results showed that all fish samples from the three markets were contaminated with *Staphylococcus aureus*. Of the three markets affected by S. aureus, the Lobia market was the most contaminated by these pathogens. In addition, the horse mackerel fish in the Lobia market was heavily contaminated with all the fish in these different markets with an average of 0.77. 10^7 UFC/g. (Table 2)

Table 2. Level of *Staphylococcus aureus* contamination of smoked fish

Market / Fish	Lobia	Orly	Big Market
Horse mackerel	$0,77.10^{7}\pm 0,13.10^{8\,b}$	$0,5.10^{6}\pm 0,71.10^{6\,a}$	$0,14.10^{6}\pm 0,26.10^{5\,a}$
Herring	$1,2.10^{6}\pm 0,9.10^{6\,a}$	$0,5.10^{6}\pm 0,62.10^{6\,a}$	$0,61.10^{6}\pm 0,97.10^{6\,b}$
Mackerel	$0,12.10^{7}\pm 0,1.10^{7\,a}$	$3,8.10^{5}\pm 5\,,1.10^{5\,b}$	$0,14.10^{6}\pm 0,2.10^{5\,a}$

3.3.2. Escherichia Coli

Fish samples from all three markets were highly contaminated with *E. coli*. Thus, the samples from the Lobia market were the most contaminated, unlike the two other markets, Orly and Grand Marché, there was also a massive presence of *E. coli* in the horse mackerel fish samples with an average load of $0.89.10^{5}$UFC/g, unlike the herring and horse mackerel samples from the market with respective loads of $0.66.10^{5}$ UFC/g and $9.3.10^{4}$ UFC/g (Table 3).

Table 3. Level of contamination of smoked fish with *Escherichia coli*

Market / Fish	Lobia	Orly	Big Market
Horse mackerel	$0,89.10^{5}\pm 0,77.10^{5\,a}$	$1,7.10^{4}\pm 1,9.10^{4\,a}$	$0,16.10^{5}\pm 0,23.10^{5\,a}$
Herring	$0,66.10^{5}\pm 6,2.10^{4\,b}$	$0,67.10^{4}\pm 0,31.10^{4\,b}$	$0,19.10^{5}\pm 0,24.10^{5\,b}$
Mackerel	$9,3.10^{4}\pm 0,51.10^{3a}$	$0,16.10^{5}\pm 0,18.10^{5\,a}$	$0,16.10^{5}\pm 0,2.10^{5\,a}$

3.3.3. *Salmonella* spp.

Table 4. Level of contamination of smoked fish with *samonella* spp.

Market / Fish	Lobia	Orly	Big Market
Horse mackerel	-	++	+
Herring	+	+	++
Mackerel	++	-	++

(++): Massive presence; (+): Attendance ; (-): Absence

The results of the investigation reveal that almost all the fish samples from the three markets were contaminated with salmonella, except for horse mackerel and mackerel from the Lobia and Orly markets, respectively. In addition, the samples from the single market were heavily contaminated with these germs, unlike the other two markets. At the Lobia market, samples of mackerel fish (++) were highly contaminated with salmonella than those of herring (+) and absent in horse mackerel (-), while in the Orly market, samples of horse mackerel (++) and herring (+) fish were highly contaminated by salmonella and absent in those of mackerel (-) (Table 4).

4. Discussion

The survey carried out in the markets of Daloa made it possible to identify the main smoked fish consumed by the populations, namely horse mackerel, herring and mackerel. These products are appreciated for their richness in proteins, fatty acids and vitamins. The study revealed that 98.75 % of smoked fish traders are women, compared to only 1.25 % men, confirming the strong feminization of the sector. The majority of them are illiterate (85 %), with only 10 % having reached the first cycle and 5 % secondary school, which limits their ability to understand the microbiological risks associated with their practices. In addition, only 7.5 % have received hygiene training, which increases the risk of product contamination. These results are consistent with those of studies carried out in Togo and Côte d'Ivoire, where the processing and marketing of smoked fish is exclusively female [20,21]. The majority age group is 20 to 30 years old, and for many, this activity is the main source of income, [6,29]. These results are comparable to those of [18]. who found 80 % of women in the smoking sector, but differ from those of [27]. in Benin where the activity is almost exclusively occupied by illiterate women. The high proportion of illiteracy (85 %) observed in Daloa is consistent with the observations of [20]. The lack of hygiene training remains a concern, as fish is a highly perishable commodity and must be processed to strict standards to ensure consumer safety. The combustion of the wood and by-products used generates carcinogenic polycyclic aromatic hydrocarbons, such as benzopyrene, recognized by the WHO [11]. Smoke and heat also lead to the risk of respiratory and eye diseases in smokers. Microbiological analyses of 27 samples of smoked fish from three markets (Lobia, Orly, Big Maket) show widespread contamination by *Staphylococcus aureus*, *Salmonella* spp. and *Escherichia coli*, except for a few cases of absence of Salmonella in horse mackerel and mackerel from Lobia and Orly. These results are consistent with the work of [32]. and [31]. in Burkina Faso and Chad, who also found strong *S. aureus* contamination This presence is explained by the numerous post-smoking manipulations, transport and storage in poor conditions. The difference with the results of [17] or [19], which did not find S. aureus, could be related to the fish species analyzed and smoking practices. Horse mackerel was found to be particularly contaminated with S. aureus, reflecting a lack of hygiene and confirming the results of [20], in Benin. Poor packaging, storage and transport practices increase the risk of food poisoning, as highlighted [22]. The absence of *Salmonella* spp. in some samples could be explained by microbial competition or low water activity following smoking [19,28,29]. These results are also consistent with those of [23-26], who had shown the absence of Salmonella in smoked fish from

Benin. E. coli contamination was observed in several samples, contrary to the results of [20], but in agreement with those of [26].This is likely related to non-compliance with good hygiene practices and the use of inappropriate well water or packaging equipment. In addition, the often insufficient smoking conditions do not allow a complete reduction of the microbial load, thus promoting bacterial proliferation. The average bioburden of *S. aureus* was 45.6 % of the samples analyzed, a rate much higher than those reported by [21] and [24]. The presence of *S. aureus* is of particular concern because this bacterium produces enterotoxins responsible for foodborne illnesses characterized by vomiting, diarrhea and abdominal pain [16,30,33]. Our results, showing a high level of contamination, reflect a major health risk for consumers. In summary, this study reveals a high level of involvement of women in the fish smoking sector in Daloa, but also a low level of hygiene education and training. Microbiological analyses reveal a worrying contamination by *S. aureus*, *E. coli* and *Salmonella* spp., linked to traditional smoking and storage conditions. Controlling these risks requires better training for processors, improved infrastructure and compliance with hygiene standards to ensure consumer safety, [34,35,36].

5. Conclusion

This investigation emphasizes the microbial risks associated with smoked fish commonly marketed in Daloa, particularly herring (*Clupea harengus*), horse mackerel (*Trachurus trachurus)*, and Atlantic mackerel (*Scomber scombrus).* The findings demonstrate that although smoking lowers microbial loads, it does not ensure the elimination of spoilage or pathogenic bacteria. Species-specific variations observed in contamination levels highlight the influence of both intrinsic factors and processing practices. Post-processing handling and market conditions emerged as critical points where contamination may occur. These results reinforce the need for improved hygiene protocols throughout the production and distribution chain. Establishing routine microbiological surveillance, coupled with training of local processors and vendors, would enhance product safety and consumer protection. Beyond food safety concerns, the study also provides baseline data that could guide regulatory authorities in developing standards for smoked fish. Taken together, the outcomes underline the importance of integrating good manufacturing practices and awareness programs to improve the microbiological quality of smoked fish in local markets.

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Author Contribution Statement

All authors contributed significantly to this work. Coulibaly Ibourahema designed the study, collected the samples, and performed the microbiological analyses. Adja Massagna Coulibaly assisted in data interpretation and provided critical revisions to the manuscript. Foba Foba Isaac stephane contributed to statistical analysis and drafting of the results. Kra kouassi Athanase and Ahossi Konan Kouakou participated in the literature review and preparation of figures and tables. Konaté Ibrahim supervised the research, provided overall guidance, and reviewed the final version of the manuscript. All authors read and approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

List of Abbreviations and Symbols

APC: Aerobic Plate Count
CFU: Colony Forming Unit
DNA: Deoxyribonucleic Acid
E.: Escherichia
FAO: Food and Agriculture Organization
HACCP: Hazard Analysis and Critical Control Point
ISO: International Organization for Standardization
LAB: Lactic Acid Bacteria
MPN: Most Probable Number
PCR: Polymerase Chain Reaction
pH: Hydrogen Potential
RNA: Ribonucleic Acid
S.: Staphylococcus
SPC: Standard Plate Count
TPC: Total Plate Count
TVB-N: Total Volatile Basic Nitrogen
WHO: World Health Organization

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